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A SYNDROME OF MEGALOBLASTIC ANEMIA, IMMUNODEFICIENCY, AND EXCESSIVE NUCLEOTIDE DEGRADATION

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INTRODUCTION

Several defects of purine and pyrimidine metabolism have been associated with behavioral abnormalities. The most common and best studied is Lesch-Nyhan syndrome[1], with its characteristic aggressive, self-mutilating behavior. Deficiency of adenylosuccinate lyase[2] has been reported to be associated with infantile autistic behavior. Autistic behavior as well as seizures have been associated with thymine-uraciluria[3]. We report here a syndrome which involves behavioral abnormalities, seizures, and macrocytic anemia and which is associated with increased degradation of purine and pyrimidine nucleotides.

CASE HISTORY

The patient, a three-year-old white female, was first seen because of recurrent infections, developmental delay, and seizures. Upon examination, she was found to have mild immunodeficiency, macroycytic anemia, ataxia, and alopecia. Physical development and speech were notably delayed. IgG was borderline to low, and MCV was variable from 90 to 100 (normal for age and sex <88). A severe, recurrent sinus infection required surgical drainage. No abnormalities of amino acid or organic acid metabolism were identified by a routine metabolic screen and amino acid analysis. No unusual compounds were detected in plasma or urine by HPLC. All parameters of folic acid and B12 metabolism were found to be within normal limits. The most striking feature of her phenotype was her bizarre, and often aggressive behavior. She was hyperactive, with a short attention span, inappropriate verbalizations, and poor interaction with other children. Aggressive behavior took the form of pinching or scratching others, or biting toys. She would sometimes bang her head or poke at her eye with her finger. She had 2-3 seizures of 1-2 min duration per day. Initially, she was treated with IgG, folinic acid, depakote, and tegritol. Her sinus infection resolved and seizure activity decreased but there was no change in her MCV, behavior, or speech development. At this time, an investiga-

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tion of her nucleotide metabolism in cultured fibroblasts was begun. Based on the findings of these studies, a trial with oral nucleotides was begun. Upon initiation of this treatment, an almost immediate improvement in speech, behavior, and cognitive function was seen. Speech became more understandable and appropriate and she seemed to pay more attention to her surroundings, and focus better on tasks. Her interactions and play with other children became appropriate, and her mother described her behavior as that of a normal child. MCV remained elevated. Seizure activity decreased markedly, such that she was taken off depakote (625 mg/day), and the dose of tegritol was gradually reduced from 500 to 50 mg/day, with the intention of eliminating it as well. However, an interuption in the supply of nucleotides caused a one week interuption in oral nucleotide therapy. During this time, seizure activity increased to >10 seizures per day. Her attention span became limited, and her frustration tolerance low. Verbalization and interaction with others deteriorated, and behavior became more aggressive. At that time she was returned to pretreatment doses of depakote and tegritol. Upon resumption of nucleotide therapy, these symptoms resided, and her condition prior to the interuption of therapy gradually returned.

MATERIALS AND METHODS

Incorporation studies were done as described earlier[4] for adenine, guanine, hypoxanthine, formate, uridine, and thymidine. For glycine and orotic acid incorporation studies, isotope (10 uCi/ml) was added to Minimal Essential Medium and cells were grown in 75 mm plates for 72 hr, harvested by trypsinization, and analyzed by HPLC as above. Incorporation experiments were done in duplicate. For the assay of individual enzymes, cultured fibroblasts were harvested in the log phase of growth and lysed at a concentration of approximately 1 mg/ml in a 0.10 sodium phosphate buffer, pH 7.2 containing 0.05 M magnesium chloride. Serial dilutions of this lysate were incubated with a 10 uM concentration of radiolabeled substrate for 1 hr at 37°C. For the assay of UMP synthetase 1 mM PRPP was added. For the assay of uridine kinase, 1 mM ATP was added. The assays were deproteinized and analyzed by HPLC. Assays were done in triplicate. Reported values do not necessarily represent maximum enzyme activities due to the low substrate concentration. Nucleoside inhibition of erythroid colony formation by bone marrow cells was done as described earlier[5].

RESULTS AND DISCUSSION

The incorporation of purine and pyrimidine precursors into nucleotides is shown in Table 1. Normal incorporation of glycine into purines, as well

Table 1. Incorporation of Precursors into Nucleotides

Precursor	Patient	Controls (n)
adenine	8160	9727 (4)
hypoxanthine	3308	2849 (4)
guanine	3392	3129 (4)
formate + AICAR	3688	3658 (4)
glycine	10071	8350 (2)
uridine	3469	8511 (4)
thymidine	1223	1027 (2)
orotic acid	5694	18315 (2)

Incorporation is reported in units of pmol/100 nmol purines/2 hr

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as normal excretion of uric acid by the patient indicate normal denovo purine synthesis. From the incorporation of adenine, hypoxanthine, and guanine into the various purine nucleotides it is clear that the activities of enzymes of purine nucleotide interconversion (i.e. adenylosuccinate synthetase, adenylosuccinate lyase, AMP deaminase, IMP dehydrogenase GMP, synthetase, GMP reductase, and the purine nucleotide mono- and diphosphate kinases) are comparable to those of normal controls (data not shown). Similarly, the production of normal amounts of UTP, CTP, and TTP from uridine indicates that the enzymes of pyrimidine nucleotide interconversion (i.e. CTP synthetase, thymidylate synthetase, and the pyrimidine mono- and diphosphate kinases) are comparable to normal controls. The only notable differences in these precursor incorporation studies was the low incorporation of orotic acid and uridine into pyrimidine nucleotides. This could reflect low activities of the synthetic enzymes or increased catabolism of the nucleotide products.

To study this question further, individual enzyme activities in dialyzed fibroblast lysates were measured (Table 2). To determine if a deficiency existed in the synthesis of pyrimidine nucleotides, the activities of UMP synthetase and uridine kinase were measured, and found to be normal. The only consistent difference between the patient and normal controls was a ten- to thirty-fold increase in the catabolism of UMP. When the rate of purine nucleotide catabolism was measured for comparison a similar elevation was found. Interestingly, the increased catabolism of purine nucleotides had no noticeable effect on the incorporation of purine precursors into purine nucleotides, whereas the increased catabolism of pyrimidine nucleotides appeared to result in a net decrease in pyrimidine nucleotide synthesis, as measured in cultured fibroblasts. To further study the metabolism of pyrimidine nucleotides in intact cells, the effect of pyrimidine nucleosides on erythroid colony formation was measured in the presence and absence of nucleosides (Table 3). Clearly, the patient's bone marrow cells show much less inhibition of colony formation in presence of thymidine and uridine than control bone marrow cells. This indicates that in the patient's cells, pyrimidine nucleosides are either transported or phosphorylated more slowly, or that the pyrimidine nucleotides, once formed, are degraded more rapidly. Again, it is interesting to note that this effect is present with thymidine, although intact fibroblasts show no decrease in the incorporation of thymidine into thymidine nucleotides.

On the basis of these results, it was decided to intiate pyrimidine nucleotide replacement therapy. In orotic aciduria, in which there is a known defect in pyrimidine synthesis associated with macrocytic anemia, pyrimidine nucleotide replacement therapy has been quite successful. The patient was started with 150 mg/kg/day each of UMP and CMP Plasma uri

Table 2. Activities of Catabolic Enzymes in Dialyzed Cell Lysates

Enzyme (substrate)	Patient	Controls (n)
5'Nucleotidase (UMP)	7.44	0.65 (4)
5'Nucleotidase (AMP)	9.64	0.80 (4)
Nucleoside Phosphorylase (uridine)	0.41	0.44 (4)
Nucleoside Phosphorylase (inosine)	5.72	4.24 (4)
Adenosine Deaminase	3.54	5.09 (4)
UMP Synthetase	1.56	2.17 (4)
Uridine Kinase	2.86	3.51 (2)

Enzyme activities are in nmol/min/mg protein

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Table 3. Inhibition of Erythroid Colony Formation by Nucleosides

Nucleoside (concentration)	Patient	Control
None	347 (100%)	169 (100%)
Uridine (10 uM)	322 (94%)	166 (98%)
Uridine (50 uM)	320 (94%)	164 (97%)
Uridine (75 uM)	335 (98%)	56 (33%)
None	508 (100%)	328 (100%)
Thymidine (10 uM)	486 (95%)	251 (76%)
Thymidine (50 uM)	298 (59%)	0 (0%)
Thymidine (75 uM)	96 (18%)	0 (0%)

Colony formation in units of average colonies per plate

dine and erythrocyte UTP were monitored during therapy. Plasma uridine was undetectable before therapy and stayed in the range of 20-50 uM during therapy. Erythrocyte UTP was similarly undetectable before therapy, and was maintained in the range of 20-60 nmol/ml packed red cell during therapy. A general improvement in the patient's condition was noted, but MCV remained abnormally high. Because increased catabolism of purine nucleotides was also indicated, 75 mg/kg/day AMP was included, and the daily dose of pyrimidines was increased to 500 mg/kg/day. These measures produced no additional improvement, and MCV remained high.

At present, the precise metabolic basis of these symptoms remains unknown. The increase in nucleotidase activity could be the primary defect, or it could be a response to abnormal amounts of some as yet unidentified nucleotide. The fact that MCV remained high, even during nucleotide replacement therapy with adenine, cytidine, and uridine nucleotides might indicate a shortage of other nucleotides, perhaps deoxynucleotides. Alternatively, the amount of nucleotides used here may have been inadequate to maintain normal nucleotide levels in the presence of increased nucleotidase activity.

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